DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** didecyldimethylammonium chloride (DDAC) **PC Code No.:** 069149 /069208

2. **TEST MATERIAL:**[1] Bardac 2280 **Purity:** 81.0 % ai
   ID No.: 100033 : 99.88% radiochemical purity

3. **CITATION**

Author:	Henry O. Krueger, Ph.D. (Study Director), D. Desjardins, T. Kendall, and R. Vanhoven
Title:	Bardac 2280: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum)*
Study Completion Date:	June 26, 2002
Laboratory:	Wildlife, International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Sponsor:	Lonzagroup (Lonza Inc.) 17-17 Route 208 Fairlawn, New Jersey 07410
Laboratory Report ID:	Wildlife International, Ltd. Study No. 289A-152
DP Barcode:	D294163
MRID No.:	458964-02

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist, US EPA/AD/RASSB

   Signature: Kathryn V. Montague **Date:** 12/15/04

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader, US EPA/AD/RASSB

   Signature: **Date:** 12/16/04

6. **STUDY PARAMETERS**

   Definitive Test Duration: 96-hr
   Type of Concentrations: Nominal and Mean Measured (initial)

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[1]Nonlabeled and radiolabeled forms used

7. CONCLUSIONS

An algal toxicity test was performed on the freshwater alga, *Selenastrum capricornutrum*,. The effects of Bardac 2280 on algal cell density and area under the growth curve were determined for a period of 96 hours, during which the cell density in each test solution was measured using a hemacytometer and microscope every 24 hours. Effects were reported as EC_{10}, EC_{50}, EC_{90} and NOEC values based on cell density and area under the growth curve. The study results were based on the initial mean measured concentration of the test solutions. The test substance had an algistatic effect. No deformed or abnormal cells were observed.

Cell Density (µg a.i./L)	Reported (0-hr meas. conc.)	Verified (96h meas. conc., from Appendix 4.8 of study report)
24-hr		
EC_{10} (95 %CI):	3.5 (1.1 - 12)	1.41(0.2 - 3.2)
EC_{50} (95 %CI):	45 (28 - 72)	23.37 (13.8 - 52.9) slope = 1.04
NOEC:	3.3	not achieved (<2.3)
48-hr		
EC_{10} (95 %CI):	8.0 (4.3 - 15)	7.39 (2.2 - 11.8)
EC_{50} (95 %CI):	30 (22 - 41)	19.57 (12.4 - 32.7) slope = 3.00
NOEC:	14	8
72-hr		
EC_{10} (95 %CI):	11 (8.2 - 16)	7.10(1.1 - 11.5)
EC_{50} (95 %CI):	27 (22 - 32)	15.47 (8.5 - 27.0) slope = 3.75
NOEC:	14	8
96-hr		
EC_{10} (95 %CI):	14 (9.0 - 21)	6.56 (1.18 - 10.54)
EC_{50} (95 %CI):	26 (21 - 33)	14.22 (7.89 - 24.17) slope = 3.78
NOEC:	14	8

8. ADEQUACY OF THE STUDY

A. **Classification:** Core

B. **Rationale:** Although concentrations of the test chemical declined over the 96-hour study due to binding of the chemical to the test vessels, 96-hour measured concentrations were obtained, and those values were used by the Agency to determine the statistical endpoints for this study.

C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS

- The study was conducted using the Wildlife International, Ltd protocol which is based on OECD Guideline 201, harmonized OPPTS Test Guideline 850.5400, and EC Guideline L383A - C.3. The OECD and EC Guideline criteria may differ from the OPPTS Guideline (850.5400) that was used in preparing this Data Evaluation Record.

- The study was conducted in compliance with FIFRA Good Laboratory Practice Standards (40 CFR Part 160) with the exception that the characterization of the radiolabelled test substance was not determined in compliance with Good Laboratory Practice Standards.

- The reported initial test pH of 7.2 to 7.3 was slightly lower than the recommended starting pH of 7.5 ± 0.1.

- Photosynthetically-active radiation was not reported.

- The age of the stock culture was not provided.

- An exploratory range-finding test was conducted; however, details were not provided in the Study Report.

- Concentrations of the test chemical in the test vessels declined throughout the test; the authors state that this was due to binding of the chemical to the surface of the test vessel, but that the chemical was still available to the algae. Measurable concentration of the test chemical were obtained from water samples at 96 hours; those values will be used by the Agency to determine the toxicity endpoints for this study for use in risk assessments.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Selenastrum capricornatum (Raphidocelis subcapitata)* • *Skeletonema costatum* • *Anabaena flos-aquae* • *Navicula pelliculosa*	• *Selenastrum capricornatum*
Initial Number of Cells • 10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* • 77,000 cells/mL (*Skeletonema*)	• Approximately 10,000 cells/mL
Stock Culture • 3 to 7 days old	• Age not provided
Nutrients • Standard formula (ASTM E1218-20) • pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) • Freshly prepared	• Algal cells cultured and tested in freshwater algal medium (ASTM 1218-90E) • Stock nutrient solutions prepared by mixing reagent-grade chemicals with purified well water. The nutrient solutions then added to purified well water (NANOpure® water). • pH 7.5 ± 0.1 (adjusted prior to use with 10% HCL and sterilized by filtration).

B. Test System

Guideline Criteria	Reported Information
Solvent • Upper limit - 0.5 mL/L	• Solvents were not used.

Guideline Criteria	Reported Information
Temperature • 24° ± 2°C (*Selenastrum, Navicula, Anabaena*) • 20° ± 2°C (*Skeletonema*) • Recorded hourly	• Approximately 23.2 to 24.4° C. • Measured continuously in the environmental chamber and recorded twice daily in a container of water adjacent to test chambers.
Light Intensity • 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) • 2.2 K lx (± 10%) (*Anabaena*) • Photosynthetically active radiation approx. 66.5 ± 10% μEin/m^2/sec	• 3,860 to 4,730 lux (measurements taken at five locations surrounding the test flasks). • Photosynthetically active radiation not reported.
Photoperiod • 14-hr light/10-hr dark (*Skeletonema*) • Continuous (*Selenastrum, Navicula, Anabaena*)	• Continuous - 24-hr light/0-hr dark.
pH • 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaema*) • 8.1 ± 0.1 (*Skeletonema*) • Measured at beginning and end of test	• pH = 7.2 to 7.3 (0-hr). • pH = 7.5 to 8.7 (96-hr).
Oscillation Rates • 100 cycles/min (*Selenastrum*) • 60 cycles/min (*Skeletonema*)	• Maintained at 100 rpm
Test Containers • 125-500 mL Erlenmeyer flasks • Cleaned/sterilized (solvent and acid) and conditioned • Test solution volume ≤ 50% of flask volume	• 250 mL Erlenmeyer flasks pretreated with Bardac 2280 solution of each respective treatment and plugged with foam stoppers. • 100 mL test solution (<50% of flask volume).

Guideline Criteria	Reported Information
Dilution Water • Sufficient quality (e.g., ASTM Type I) • Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• Purified well water (NANOpure® water)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Water solubility and physical-chemical properties of test chemical determined? • Validated analytical method developed? • Lowest dose at detection limit, upper dose at saturation concentration or 1000 mg/L • If < 50% reduction in growth at highest dose, no definitive test required	• Unknown • A validated analytical method was developed. • Nominal test concentrations were selected in consultation with the Sponsor's Representative and were based upon the results of an exploratory range finding toxicity test. No further details were provided in the Study Report.
Dose Range • 1.5X -2X progression	• 2X progression
Doses • 5 or more concentrations of test substance in a geometric series • > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• Six concentrations: Nominal = 4.7, 9.4, 19, 38, 75, and 150 µg ai/L. • Mean measured (initial) = 3.3, 6.9, 14, 30, 63, and 130 µg ai/L. • Percent recovery 70 to 86% of nominal. • >90% growth was inhibited (99%)
Controls • Negative and/or solvent each test • Positive - zinc chloride (periodically)	• Negative control • No positive control
Replicates Per Dose • 3 or more (4 or more for *Navicula*)	• 3 replicates per dose and controls.
Duration of Test • 96-hr	• 96-hr
Growth • Logarithmic growth (controls) by 96-hr or repeat test • 1.5 x 10^6 cells/mL (*Skeletonema*) • 3.5 x 10^6 cells/mL (*Selenastrum*)	• Logarithmic growth in control by 96-hr • Mean of 2.7 x 10^6 cells/mL at 96-hr. in the control. • Increase by factor of 270.

Guideline Criteria	Reported Information
Daily Observations?	• Yes
Method of Observations • Direct - microscopic cell count of at least 400 cells/flask • Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count • Qualitative and descriptive	• Cell counts were performed using an electron particle counter (Coulter Electronics, Inc.). • Cells examined microscopically for atypical morphology. • Growth of cells were assessed for aggregations or flocculation of cells and adherence of cells to the test chamber.
Cell Separation • Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) • Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	• Rotary shaking.
Algistatic and algicidal effects differentiated?	• The 63 and 130 µg ai/L treatment groups was maximally inhibited at the end of the 96-hour exposure period. At 96 hours, 0.5 mL aliquots of the test solutions were diluted in 100 mL of culture medium and growth was observed. After six days algal growth was sufficient to indicate that the cells had recovered from the effects of the test substance; therefore, effects were found to be algistatic, rather than algicidal.
Maximum Labeled Rate	• Not reported.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	• Yes • Original algal cultures obtained from the University of Toronto Culture Collection of Algae and Cyanobacteria and maintained at Wildlife International, Ltd.
Growth in controls reported?	• Yes
Description of test system and test design included?	• Yes
Initial and final chemical concentrations and pH measured?	• Yes
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	• Yes • Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	• Yes
Raw data included?	• Partial
Methods and data records reported?	• Partial
Statistical Analysis • Mean and standard deviation calculated and plotted? • Goodness-of-fit determined?	• Only mean calculated and plotted. • Yes

Dose Response

Cell Density (cells/mL)

Mean Measured Concentration at Test Initiation (µg ai/L)	24 -hour		48-hour		72-hour		96-hour	
	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]
Control	35,601	---	142,293	---	720,241	---	2,707,910	---
3.3	30,265	15	144,561	-1.60	736,352	-2.2	2,733,051	-0.93
6.9	26,709*	25	143,393	-0.77	680,655	5.5	2,530,494	6.6
14	27,805*	22	131,303	7.7	698,989	3.0	2,496,981	7.8
30	21,180*	41	66,703*	53	298,086*	59	1,127,727*	58
63	13,416*	62	28,984*	80	50,490*	93	56,842*	98
130	10,694*	70	16,231*	89	17,830*	98	25,206*	99

a) Percent inhibition was calculated relative to the negative control replicates using SAS Version 8.
* Statistically significant difference ($p<0.05$) from the negative control replicates using Dunnett's Test.

Mean Area Under the Growth Curve

Mean Measured Concentration at Test Initiation (µg ai/L)	24 -hour		48-hour		72-hour		96-hour	
	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]
Control	307,212	---	2,201,936	---	12,312,336	---	53,210,140	---
3.3	243,180	21	2,101,096	4.6	12,432,056	-0.97	53,824,888	-1.2
6.9	200,508*	35	2,001,728	9.1	11,650,304	5.4	49,944,092	6.1
14	213,664*	30	1,882,964	14	11,606,468	5.7	49,718,112	6.6
30	134,160*	56	948,760*	57	5,086,228*	59	21,955,984*	59
63	40,992*	87	309,796*	86	1,023,492*	92	2,071,484*	96
130	16,528*	95	99,624*	95	268,356*	98	544,788*	99

a) Percent inhibition was calculated relative to the negative control replicates using SAS Version 8.
* Statistically significant difference ($p<0.05$) from the negative control replicates using Dunnett's Test.

Statistical Results

Statistical Method: Cell density and area under the growth curve were analyzed statistically by non-linear regression versus concentration to determine EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits for each 24-hour exposure interval. In cases where EC_{50} values could not be determined by non-linear regression, EC values and 95% confidence limits were calculated by linear interpolation versus concentration using TOXSTAT Version 3.5. To determine the NOEC, cell density and the area under the growth curve data were first evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively, and were compared to the negative control using analysis of variance (ANOVA) and Dunnett's test.

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Cell Density Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	3.5 (1.1 - 12)	45 (28 - 72)	>130 (NA)	3.3
48-hr	8.0 (4.3 - 15)	30 (22 - 41)	113 (88 - 146)	14
72-hr	11 (8.2 - 16)	27 (22 - 32)	63 (54 - 72)	14
96-hr	14 (9.0 - 21)	26 (21 - 33)	51 (42 - 62)	14

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Area Under the Growth Curve Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	7.0 (2.9 - 17)	26 (16 - 41)	96 (67 - 137)	3.3
48-hr	9.3 (5.7 - 15)	27 (21-35)	81 (67 - 99)	14
72-hr	11 (8.0 - 16)	27 (22 - 32)	64 (55 - 74)	14
96-hr	13 (9.2 - 18)	26 (22 - 32)	54 (46 - 63)	14

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

NOEC Determination
The data were first checked for normality using the Shapiro-Wilks' Test. All data were normally distributed. Therefore, the NOECs were determined using Dunnett's Test.

EC_{10}, EC_{50}, and EC_{90} Determination
The EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits were calculated for cell densities. EbC_{50} and ErC_{50}, as well as 95% confidence intervals were also determined for biomass and growth rate. The EC values were determined using EPA's Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach.

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Cell Density Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	2.2 (0.3 - 16)	44 (26 - 75)	>130 (NA)	3.3
48-hr	15 (-9.7 - 20)	29 (24 - 36)	>130 (NA)	14
72-hr	15 (-4 - 18)	27 (24 - 30)	60 (57 - 63)	14
96-hr	15 (-4.7 - 17)	27 (24 - 32)	56 (54 - 59)	14

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Area Under the Growth Curve Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	1.6 (0.27 - 8.8)	26 (12 - 39)	91 (22 - 159)	3.3
48-hr	8.1 (-4.6 - 23)	27 (23 - 30)	92 (31 - 136)	14
72-hr	15 (-5.3 - 18)	27 (24 - 30)	61 (58 - 68)	14
96-hr	15 (-4.4 - 17)	27 (24 - 31)	58 (55 - 59)	14

14. REVIEWER'S COMMENTS:

- Verified NOEC values are the same as reported in the Study.
- Verified EC_{50} values are the same or are very similar to the those reported in the Study. Differences may be due to the statistical method used.
- At the 96-hour interval, the mean measured concentrations based on liquid scintillation counting (LSC) were 49, 43, 42, 50, 36, and 51 percent of nominal in the 4.7, 9.4, 19, 38, 75, and 150 µg/L treatment groups, respectively. Some of the DDAC may have bonded to the glassware. However, according to the Study Report, DDAC bound to the glass surfaces of the glassware remains biologically available to algae in the test system.